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DIFFERENTIAL STAINING OF SECTIONS OF UNPRESERVED BOVINE UDDER TISSUE AFFECTED WITH MASTITIS

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INTRODUCTION

In the normally functioning quarter of a bovine udder, the glandular tissue constitutes the major structure of the organ and is supported by a connective-tissue framework. Little evidence of this supporting tissue is observed in the upper two-thirds of the quarter except as fine lines radiating through the glandular tissue and around the milk ducts and blood vessels. Around the cistern and in the lower third of the quarter, there is a normal increase in the quantity of connective tissue, owing to the presence of the larger milk ducts leading to the cistern. When a section is cut to include both the front and rear quarters of one-half of the udder, other tissues such as fat, skin, and lymphoid tissue from the supramammary lymph gland are also present, all of which contain a certain proportion of connective tissue.

It is believed that in most cases of mastitis, infection enters the quarter by way of the teat canal and establishes itself in the cistern. As the infection extends from this point to other parts of the gland, its progress is accompanied by a proliferation of connective or scar tissue at the expense of the secreting tissue. A typical lesion, therefore, consists in the replacement of the normal glandular tissue by connective or scar tissue as a result of irritation from a low-grade chronic infection. This infection is caused usually by streptococci or other species of cocci, although other types of bacteria may be present. When a quarter of the udder has been invaded by cocci, the infection persists as a rule for the remainder of the useful life of the animal. A few animals seem able to overcome the infection, but when it has been present for any appreciable length of time the physical evidence of its sojourn can still be detected by palpation of the organ.

The problem of demonstrating the gross changes that occur in the bovine udder as a result of chronic mastitis has presented some difficulty. As a result of invasion of the gland by pyogenic organisms, abscesses and purulent foci occur in a small percentage of quarters affected with mastitis. Lesions of this type are readily recognized, but they are not representative of the usual conditions found in diseased quarters. Unfortunately the characteristic indurative changes that are found in most quarters so affected do not present similar outstanding features. Consequently, considerable training and experience are required to distinguish macroscopic alterations in the udder tissue at autopsy, especially when the changes are not extensive.

To study the extent of induration in the organ and to enable the untrained observer to detect the increased connective or scar tissue present in quarters affected with mastitis, the writers have developed a differential staining technique for demonstrating connective tissue in the unfixed bovine udder. Sections stained by this method photograph well for making colored lantern slides.

SECTIONING THE UDDER

The udder is separated into halves and each half placed in a freezing compartment at a temperature of 0° F. To prevent distortion of the tissue in relation to the teats, the specimen should be placed in a flat tray with the median septum down. A period of 18 to 24 hours at this temperature is usually long enough to insure the sections being frozen through. At the end of this time it should be allowed to thaw slowly at a temperature of about 35° for 24 hours. When the tissue has thawed completely, sections from ½ to 1 inch thick with smooth regular surfaces can be cut from it with a long sharp knife. By thawing the udder in this manner no ice remains in the milk ducts to interfere with sectioning, and the tissue remains sufficiently firm to be cut evenly. Fresh, unfrozen udder tissue is too soft to have a smooth surface when sectioned.

The tissue is then placed in cold running water so that as much milk as possible will be washed out. The time required for washing varies, depending on the thickness of the section and the quantity of milk present, but the washing should be continued until the fluid expressed with light pressure is no longer cloudy. If any milk remains, the final solution in which the specimen is immersed for study or photographing will become hazy and the structural details will be obscured.

STAINING THE SPECIMEN

After the tissue has been thoroughly washed it is drained for several minutes and immediately placed in a 1 in 2,000 aqueous solution of acid fuchsin.[1] The solution is made by dissolving 1 g of dry dye in 2,000 cc of cold tap water. Sufficient dye solution should be used to cover the whole section completely. The time required for staining is variable, but 5 to 10 minutes is usually satisfactory. How-

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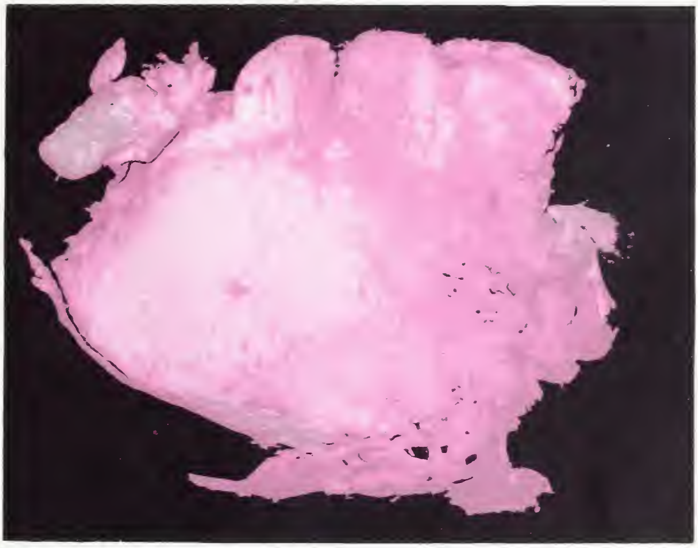
[1] Product of the National Aniline & Chemical Co., Inc.



Longitudinal section of half of a bovine udder. Cut made through the center of the front (right) and rear (left) quarters. The front quarter is almost completely indurated, as indicated by the deeper red color, and is somewhat atrophied. The teat has been removed from this quarter. The rear quarter, which constitutes about two-thirds of the section, has some induration (red color) around the cistern. The rest of the quarter is composed of more or less normal glandular tissue (unstained portion). The teat of this quarter is found in the lower right-hand corner and the supramammary lymph gland in the upper left-hand corner.

ever, it is advisable to remove the tissue from the staining solution after 2 to 3 minutes and wash in cold running water to observe the progress of the stain. At this time the connective tissue will be perceptibly red and the glandular tissue a faint pink. The section should next be returned to the staining solution for another 2 to 3 minutes and then reexamined as before. When the glandular tissue begins to acquire a definitely reddish shade, staining should be stopped by transferring the specimen to cold running tap water.

This washing should continue until the connective tissue assumes a dull pink shade. Any dye taken up by the normal glandular tissue will have been completely removed by this time. The section is then placed in a 0.1-percent solution of glacial acetic acid in cold tap water. In this medium, the bright red color is restored to the connective or fibrous tissue.

In affected quarters that are not completely indurated, the lobes of normal glandular tissue remain unstained, whereas other lobes, which appeared normal prior to staining, have developed a faint pinkish shade, and the frankly indurated portions have acquired the characteristic red color of connective tissue. It is probable that the pink coloring of the otherwise normal-appearing lobes of glandular tissue may be indicative of an early invasion by connective or scar tissue.

APPEARANCE OF THE STAINED SPECIMEN

Plate 1 illustrates the distribution of connective tissue in one-half of an udder, in one quarter of which the lesions of mastitis are very extensive, whereas relatively little change has occurred in the other quarter. The rear quarter (left), to which the teat is attached, can be readily identified by the section of supramammary lymph gland that appears in the upper left-hand corner. In this quarter, there is an area of fibrosis around and immediately above the cistern that is rather sharply demarcated from the normal unstained secreting tissue of the gland. Along the upper margin of the quarter and adjacent to the lymph gland is an area of fat tissue which is also unstained. The stained connective-tissue framework of both these types of tissue, as well as that of the normal glandular portion of the quarter, stands out in distinct contrast with the unstained tissue.

The front quarter, on the other hand, has only a small quantity of glandular tissue remaining in the extreme upper portion of the gland. The rest of the quarter is composed almost exclusively of connective or scar tissue which, because of its red color, distinctly delineates the line of separation between the two quarters. As a result of the advanced stage of induration of the quarter, the organ appears to be somewhat atrophied. On account of this solidification, also, the tissue apparently takes a somewhat deeper shade of red than the scar tissue about the cistern of the rear quarter.

PRESERVING THE STAINED SPECIMEN

No successful method has been found for preserving the stained specimens for an extended period of time. Consequently, they should be examined or photographed as soon as possible after preparation. The sections photograph well when submerged in 0.1-percent acetic acid solution, but it may be necessary to change the solution occasionally because of a tendency for the dye to diffuse into it. When necessary, the specimens may be held for 24 to 36 hours if they are kept cold and the acid solution changed from time to time. Specimens held for this length of time, however, cannot be restained satisfactorily.

SUMMARY

A method is described by which unpreserved sections of bovine udder tissue can be stained to facilitate examination for indications of mastitis. Intensity of staining corresponds to the degree to which the disease has developed.

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